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EVALUATION OF MOLECULAR WEIGHT FROM EQUILIBRIUM SEDIMENTATION

PART I. COMPUTATION BASED ON EXPERIMENTAL SCHLIEREN PLOT

MATATIAHU GEHATIA

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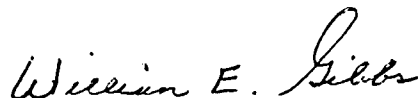## PART I. COMPUTATION BASED ON EXPERIMENTAL SCHLIEREN PLOT

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FOREWORD

This report was prepared by the Polymer Branch of the Nonmetallic Materials Division. The work was initiated under Project No. 7342, "Fundamental Research on Macromolecular Materials and Lubrication Phenomena," Task No. 734203, "Fundamental Principles Determining the Behavior of Macromolecules," and the research was conducted by Dr. M. T. Gehatia (MANP). The work was administered under the direction of the Air Force Materials Laboratory, Research and Technology Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio.

The report covers research conducted from January 1966 to February 1967. The manuscript was released by the author in February 1967 for publication as a technical report.

This technical report has been reviewed and is approved.

WILLIAM E. GIBBS
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ABSTRACT

A new method has been developed to determine molecular weight (specifically, ratio $g = s\omega^2/D$, where ω = angular velocity, s = sedimentation coefficient, D = diffusion coefficient) from an experimental schlieren plot, dc/dr vs r, taken at equilibrium. Computation of the quantity g can be accomplished by applying the newly derived formula:

$$\ln\left(\frac{1}{r}\frac{dc}{dr}\right) = \ln(gk) + \frac{1}{2}gr^2,$$

where

$$k = (c)\exp\left(-\frac{1}{2}gr^2\right)$$

is a constant, and c is concentration at distance r from center of rotation.

For a case of a polydisperse system, the following method of computation has been developed. The heterogeneous system was replaced by an equivalent N-component paucidisperse solute. A set of N $g^{(i)}$ values was arbitrarily chosen, where each $g^{(i)}$ is related to component, i, present in the system. The following expressions were defined:

$$y_j = 2/r_j(r_b^2 - r_m^2),$$

is a quantity measured at point j on the experimental plot; b and m denote bottom and meniscus, respectively; and the function

$$a_{ji} = \left[g^{(i)^2}\exp\left(\frac{1}{2}g^{(i)}r_j^2\right)\right] / \left[\exp\left(\frac{1}{2}g^{(i)}r_b^2\right) - \exp\left(\frac{1}{2}g^{(i)}r_m^2\right)\right].$$

Since it is shown that

$$y_j = \sum_{i=1}^{N} a_{ji} c_o^{(i)},$$

where $c_o^{(i)}$ is the initial concentration of component i, evaluation of a_{ji} and y_j experimentally leads directly to the determination of $c_o^{(i)}$. Therefore, by applying this method, one can determine the entire distribution of molecular weight from equilibrium sedimentation, as well as the average values.

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SECTION I

INTRODUCTION

1. EQUILIBRIUM SEDIMENTATION

Equilibrium sedimentation was first applied by Svedberg in his early works (Reference 1). Since, at equilibrium, concentration of polymers in solution does not depend upon time, the equation of ultracentrifuge reduces to an ordinary differential equation:

$$\frac{\partial c}{\partial t} = -\operatorname{div} \underline{J} = 0,$$ (1)

where

  c = polymer concentration

  $\underline{J}$ = flux of solute.

Hence

$$|\underline{J}| = |-Dc_r + s\omega^2 cr| = \text{const.}$$ (2)

D and s are coefficients of diffusion and sedimentation, respectively.

  ω = angular velocity of ultracentrifuge;

  r = distance from center of rotation

  $c_r = dc/dr.$

Since at equilibrium no flux occurs, $\underline{J} = 0$, the following formula holds:

$$D\frac{dc}{dr} = s\omega^2 cr$$ (3)

By introducing a new constant, $g = s\omega^2/D$, the Differential Equation (3) can be written in a short form:

$$\frac{dc}{dr} = grc,$$ (4)

or:

$$d(\ln c) = \frac{1}{2}gc\,d(r^2)$$ (5)

Formula (5) leads to a well known method of computation (Reference 1) which determines g from any two points i and j of an experimental plot c vs r:

$$\ln(c_i/c_j) = \frac{1}{2}g(r_i^2 - r_j^2).$$ (6)

By substituting g into the Svedberg Equation (Reference 2), molecular weight can be easily evaluated:

$$M = \frac{RTg}{(1 - V\rho)\omega^2} \, , \tag{7}$$

where

M = molecular weight

R = gas constant

T = absolute temperature in °K

V = partial specific volume of solute

ρ = density of solvent.

2. LIMITATIONS OF EQUILIBRIUM SEDIMENTATION

The following are limiting factors in the application of equilibrium sedimentation.

a. The time of the experiment becomes exceedingly long if M > 250,000. If M > 1,000,000 this method is not feasible. Therefore, equilibrium sedimentation can only be advantageously applied if molecular weight is below 250,000.

b. Schlieren optics is the simplest and most generally used experimental technique. However, this method provides a plot of dc/dr vs r. Since the conventional methods of computation require a plot of c vs r (see Equation (6)), the experimental schlieren curve has to be transformed into an integral one. Such a transformation is very laborious and not sufficiently precise.

c. A plot of c vs r can be obtained by applying interference optics. Unfortunately, this method cannot be used where high speed, elevated temperature, or corrosive solvents are involved.

d. Absorption optics also provide a plot c vs r. However, this technique requires a tedious procedure of calibration and evaluation, and very good conditions for precise photographic processing.

e. In recent years a new technique has been under development (Reference 3) which also leads to an experimental plot of c vs r. This method is based upon direct scanning of an ultracentrifugal cell by photosensitive elements. Unfortunately, this optical system is not yet complete and, in addition, necessary equipment is very expensive.

3. PRIOR ATTEMPTS TO IMPROVE EQUILIBRIUM SEDIMENTATION

Some attempts have been made to improve the method of equilibrium sedimentation and to relax its limitations.

Archibald (References 4 and 5) applied Equation (4) to evaluate g at the meniscus at any time; e.g., before equilibrium. Since no transport can exist at the meniscus and bottom of the ultracentrifugal cell, an equilibrium equation must always hold at this particular point. Some of his evaluations are of general use, and are mentioned subsequently. Experimental difficulties, however, arose which limited the application of Archibald's method.

Another method, "sedimentation at constant density gradient," was successfully developed by Meselson, et al. (Reference 6). This technique, which significantly shortens the time of an experiment, requires the presence of a special binary or multicomponent solvent. Such a system is supposed to give a constant density gradient and to cause sedimentation and flotation of polymer toward the middle of the cell. It is evident that each family of polymers can be investigated in this manner only if an appropriate solvent system has already been developed. Unfortunately, at present there are only two successful systems -- one for deoxyribonucleic acid and one for polystyrene.

Since molecular weights of polymers under current investigation in the Air Force Materials Laboratory do not exceed 250,000, conventional equilibrium sedimentation can be successfully applied. Therefore, the objective of this work is to improve the methods of computation and determine g directly from an experimental plot of c_r vs r.

SECTION II

MATHEMATICAL ANALYSIS OF EQUILIBRIUM SEDIMENTATION

Integration of Differential Equation (4) leads to the following solution:

$$c = k e^{\frac{1}{2} g r^2}, \tag{8}$$

where k is a constant

Hence:

$$k = c e^{-\frac{1}{2} g r^2} = c_m e^{-\frac{1}{2} g r_m^2} = c_b e^{-\frac{1}{2} g r_b^2} = \cdots \tag{9}$$

m denotes meniscus and b bottom of the cell.

Following Equation (9) the concentration at any point can be given as a function of any chosen reference point (c_q, r_q):

$$c = c_q e^{\frac{1}{2} g (r^2 - r_q^2)} \tag{10}$$

The first derivative of c is given by Equation (11):

$$c_r = g r c_q e^{\frac{1}{2} g (r^2 - r_q^2)} \tag{11}$$

Further differentiation of Equation (11) leads to the following relationship:

$$c_{rr} = gc_q e^{\frac{1}{2}g(r^2 - r_q^2)} + g^2 r^2 c_q e^{\frac{1}{2}g(r^2 - r_q^2)} \tag{12}$$

By combining Equations (10), (11), and (12), one will obtain:

$$c_{rr} = \left(\frac{1}{r} + gr\right)c_r \tag{13}$$

or:

$$\frac{d\,\ell n\,c_r}{dr} - \frac{1}{r} = gr \tag{14}$$

Equations (8) and (14) lead to the following expression:

$$\ell n\,c_r = \ell n\,(kgr) + \frac{1}{2}gr^2 \tag{15}$$

and finally:

$$\ell n\,(c_r/r) = \ell n\,(gk) + \frac{1}{2}gr^2. \tag{16}$$

Equation (16) can be applied for the computation of g. By plotting $\ell n\,(c_r/r)$ vs r^2, one can obtain the constant values $g/2$ and $\ell n(gk)$. With the help of g and k the concentration c can be evaluated at any r; c_m and c_b (concentrations at meniscus and bottom respectively) can be determined in the same manner.

There is another way to evaluate g by approximation in the vicinity of a chosen reference point (c_q, r_q). Since under these circumstances $1/2\,g(r^2 - r_q^2)$ is a small number, Equation (10) reduces to the following series:

$$\frac{c_r}{r} = gc_q + \frac{1}{2}g^2 c_q (r^2 - r_q^2) + \cdots \tag{17}$$

A plot of (c_r/r) vs $(1/2)(r^2 - r_q^2)$ provides values of the constants g, c_q, and $g^2 c_q$, and makes it possible to determine g and c_q. Since no flux exists at the boundaries of an ultracentrifugal cell, any equation describing equilibrium always holds at the meniscus. Therefore, the method of computation expressed by Equation (16) can be applied in the vicinity of the meniscus, and such an application can be made at any time; e.g., even before equilibrium is established. Under these conditions g (and c_m) have to be determined as limiting values for $r \longrightarrow r_m$.

SECTION III

POLYDISPERSE IDEAL SYSTEM

Let us consider an ideal dilute polydisperse system composed of N-noninteracting components. At equilibrium each component attains its own equilibrium state. Therefore, there exists a set of the following N equations:

$$D^{(i)} c_r^{(i)} = s^{(i)} \omega^2 r c^{(i)}, \tag{18}$$

where (i) denotes component i.

Or in terms of $g^{(i)} = j^{(i)} \omega^2 / D^{(i)}$:

$$c_r^{(i)} = g^{(i)} r c^{(i)}. \tag{19}$$

Solutions of the differential equations given by Formula (19) can be expressed either by constants $k^{(i)}$ or by any reference concentration $c_q^{(i)}$.

$$c^{(i)} = k^{(i)} e^{\frac{1}{2} g^{(i)} r^2} = c_q^{(i)} e^{\frac{1}{2} g^{(i)} (r^2 - r_q^2)} = c_m^{(i)} e^{\frac{1}{2} g^{(i)} (r^2 - r_m^2)} = \cdots \tag{20}$$

Another relationship can be derived from the law of conservation of matter. Since the amount of solute enclosed in a cell remains constant, the following equation holds at any time:

$$A = \int_{r_m}^{r_b} \psi c \, dr, \tag{21}$$

where A is the amount of solute in the cell, and $\psi(r)$ is the area of the cylindrical surface within the cell at the distance r from the center of rotation. In the case of a sectorial cell with angle ϕ and depth h:

$$\psi = \phi r h \tag{22}$$

and Equation (21) goes into the following formula:

$$A = \int_{r_m}^{r_b} h \phi c r \, dr \tag{23}$$

where A, h and ϕ are constant.

If c_0 denotes the initial homogeneous concentration, one can derive from Equation (23) the following relationship:

$$c_0 (r_b^2 - r_m^2) = \int_{r_m^2}^{r_b^2} c \, dr^2 \tag{24}$$

Equations (21 through 24) were first derived by Archibald (References 4 and 5).

By substituting Equation (8) into (24) one can obtain:

$$c_0 (r_b^2 - r_m^2) = k \int_{r_m^2}^{r_b^2} e^{\frac{1}{2}gr^2} dr^2 = \frac{2k}{g} (e^{\frac{1}{2}gr_b^2} - e^{\frac{1}{2}gr_m^2}). \tag{25}$$

Hence:

$$c_0 (r_b^2 - r_m^2) = \frac{2}{g} (c_b - c_m) \tag{26}$$

Equation (26) was derived in a different way by Van Holde and Baldwin (Reference 9).

The value $(c_b - c_m)$ which expresses the increase of concentration between the meniscus and bottom of the cell can be easily determined from experiment by integrating c_r between all boundaries:

$$c_b - c_m = \int_{r_m}^{r_b} c_r dr . \tag{27}$$

By substituting the value of $(c_b - c_m)$ into Equation (26) one can easily determine g.

In the following, Equation (26) will be applied to evaluate the distribution of a polydisperse system. Since Equation (26) holds for any component of a heterogeneous system, one can write:

$$c_0^{(i)} = \frac{2 \left[c_b^{(i)} - c_m^{(i)} \right]}{g^{(i)} (r_b^2 - r_m^2)} . \tag{28}$$

Equations (20) and (11), if applied at the meniscus for component i, go into the following expressions:

$$c_b^{(i)} = c_m^{(i)} e^{\frac{1}{2}g^{(i)}(r_b^2 - r_m^2)} , \tag{29}$$

and:

$$c_r^{(i)} = g^{(i)} r e^{\frac{1}{2}g^{(i)}(r^2 - r_m^2)} c_m^{(i)} . \tag{30}$$

First, the quantity $c_b^{(i)}$ will be eliminated. By combining Equations (28) and (29) the following expression will be obtained:

$$c_0^{(i)} = \frac{2}{g^{(i)}(r_b^2 - r_m^2)} \left[e^{\frac{1}{2}g^{(i)}(r_b^2 - r_m^2)} - 1 \right] c_m^{(i)} . \tag{31}$$

By eliminating the quantity $c_m^{(i)}$ from Equations (30) and (31), one may express $c_r^{(i)}$ as a function of $g^{(i)}$ and $c_o^{(i)}$:

$$c_r^{(i)} = \frac{r(r_b^2 - r_m^2)}{2} \left[\frac{g^{(i)2} e^{\frac{1}{2} g^{(i)} r^2}}{e^{\frac{1}{2} g^{(i)} r_b^2} - e^{\frac{1}{2} g^{(i)} r_m^2}} \right] c_o^{(i)}. \tag{32}$$

Formula (32) can be applied to any one of the N components present in the system under investigation. Therefore, this formula represents a set of N equations. By carrying out the summation of all N equations of Formula (32) one will obtain the following relationship:

$$c_r = \frac{1}{2} r(r_b^2 - r_m^2) \sum_{i=1}^{N} \left[\frac{g^{(i)2} e^{\frac{1}{2} g^{(i)} r^2}}{e^{\frac{1}{2} g^{(i)} r_b^2} - e^{\frac{1}{2} g^{(i)} r_m^2}} \right] c_o^{(i)}, \tag{33}$$

where $c_r = \sum_{i=1}^{N} c_r^{(i)}$ can be directly measured experimentally.

The objective of this section is to evaluate the molecular weight distribution. Since the ratio, P:

$$P = \frac{RT}{(1 - V\rho)\omega^2}, \tag{34}$$

which appears in Equation (7) is an experimental constant, identical for all components present in the system:

$$M = P g^{(i)}, \tag{35}$$

the distribution of molecular weight, $M^{(i)}$, can be replaced by a distribution of quantities $g^{(i)}$. It should be mentioned that evaluation of a distribution means an appropriate adjustment of the pairs $(g^{(i)}, c_o^{(i)})$. Such a definition gives one the freedom to arbitrarily choose sets of quantities mentioned above. This freedom is utilized in the following way:

First, a set of N $g^{(i)}$ values will be chosen arbitrarily. However, one has to bear in mind, that the experimental average g values cannot be outside of this set. In addition such a set cannot be very narrow, and it must be extended over a wide range of possible g values.

Let us now denote by j a specifically chosen point (r_j, c_{r_j}) on the experimental curve. For any chosen pair (r_j, c_{r_j}) and for any arbitrarily chosen value of $g^{(i)}$, the following quantities can be determined:

$$y_j = 2c_{r_j} / r_j (r_b^2 - r_m^2) , \tag{36}$$

and:

$$a_{ji} = \frac{g^{(i)2} e^{\frac{1}{2} g^{(i)} r_j^2}}{e^{\frac{1}{2} g^{(i)} r_b^2} - e^{\frac{1}{2} g^{(i)} r_m^2}} . \tag{37}$$

By introducing the definitions given in Equations (36) and (37), the expression cited in Equation (33) can be transformed into summation:

$$y_j = \sum_{i=1}^{N} a_{ji} c_o^{(i)} , \tag{38}$$

where y_j and a_{ji} are known quantities.

Let us choose L pairs (r_j, c_{r_j}) where $L \geq N$. Since Equation (38) holds for any one of such defined L pairs, there exists a system of L simultaneous linear equations, with N unknown quantities $c_o^{(i)}$:

$$
\begin{aligned}
y_1 &= \sum_{i=1}^{N} a_{ij} c_o^{(i)} \\
&\vdots \\
y_L &= \sum_{i=1}^{N} a_{Li} c_o^{(i)}
\end{aligned}
\tag{39}
$$

The following definitions of two vectors and one matrix:

$$\underline{Y} = (y_1 \cdots y_L) ; \tag{40}$$

$$\underline{C} = (c_o^{(1)} \cdots c_o^{(N)}) ; \tag{41}$$

$$A = \begin{pmatrix} a_{11} & \cdots & a_{1N} \\ \vdots & & \\ a_{L1} & \cdots & a_{LN} \end{pmatrix} , \tag{42}$$

make it possible to express the System indicated in Equation (39) in a compact form:

$$\underline{Y} = A\underline{C} . \tag{43}$$

If L = N one can immediately write the solution

$$\underline{C} = B\underline{Y} . \tag{44}$$

where $B = A^{-1}$ is the inverse matrix of A.

If L > N a method of least squares can be applied to evaluate vector $\underline{C}$.

Since the elements of $\underline{C}$ are correlated to the appropriate set of $g^{(i)}$ values, the evaluation of vector $\underline{C}$ accomplishes the determination of the molecular weight distribution.

The theory derived in this work is an approximate one. It disregards any influence of virial coefficient, interactions within a multicomponent system, compressibility in the ultracentrifuge, and other nonspecified factors.

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